Vitality and storage condition of *Syringa* pollen

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Abstract: The fresh pollen vitality, the effect of different storage conditions on the pollen vitality, and the difference of vitality among the species of seven species of *Syringa* were determined in Shenyang, China. The results indicated that the pollen vitality (81.5%) of *Syringa villosa* was the highest among the seven tested species, followed by *S. microphylla* and *S. meyeri*, and that of *S. oblata* var. *affinis* was the lowest. The low temperature was the best condition for storage of pollen of *Syringa*, and the most proper temperature for the storage was 0-2 °C. The storability of *S. microphylla* was the best of all, and it could be stored over 60 days at the temperature of 0-2 °C, next was *S. villosa* and *S. meyeri*.

Keywords: *Syringa*; Pollen; Vitality; Storage condition

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Introduction

The vitality and storage characteristics of fresh pollen is one of the important research contents of flowering biology (Gong 1997), in particular, for the species with different flowering periods from parent plants crossbreeding or long distance pollination. The storage and/or transportation of the pollens maybe influence the vitality of pollens and the success rate of crossbreeding in a certain extent (Wang 2002). Thus, before and after the process of storing and/or transporting the pollens, and before hybridization, the vitality of pollen should be tested. Many researches have indicated that the vitality and storage conditions of pollen of different species are obviously different even under the same natural conditions (Wang 2002; Li 1999). And low temperature can prolong the life of pollen (Xue 2000; Zhang 2000). These results have practical significance to the studies on the interaction between pollen and chapiter, the conservation of gene pool, and on the relationship between the fertilization and germination of pollens (Lai 1994). If we sufficiently understand the characteristics of vitality and storability of the pollen about the correlative plants, we could select the parent plants accurately and set up the hybrid combination reasonably to improve the success rate of hybridization, and then analyze the hybrid results scientifically.

Lilacs *(Syringa* spp.*)* are famous ornamental shrubs from osmanthus *(*Oleaceae*)* (Zang 1990) because of their elegant and fragrant features (Wu 1998). *Syringa* spp. have been successfully cultivated for over 1 000 years in the world (Zang 1993). China is their native territory and distributing center. Of thirty-two *Spring*a species in the world, twenty-seven species (about 80%) are widely distributed in China (found in 15 provinces) (Zang 1992). The abundant genetic resources offer a favorable condition for the crossbreeding within *Syringa* (Liu 2000; Zhao 1999). However, the crossbreeding between species of *Syringa* is often very difficult due to the wide geographical range and various ecological characteristics. Thus, before crossbreeding, fully understanding and mastering the characteristics of pollens of *Syringa* become very important, particularly for the needs of practice, scientific research, and the conservation of genetic resource. In recent years, a few of researches mainly focused on the testing method of the pollen vitality and the stored length of pollen (Gu 1998; Liang 2000). In this paper, we presented the study results on the variation of pollen vitality of different species of *Syringa* under different storage conditions. Moreover, we adopted different methods to defrost the pollen after the pollen being frosted or frozen in order to discover which method was the best for storing *Syringa* pollen and which species could be used as suitable parent plants for the crossbreeding in different regions.

Material and methods

Material

The pollens of 7 species of *Syringa* separately came from the arboretums of Shenyang Institute of Applied Ecology and Shenyang Agricultural University. The species are as follows:

(1) *Syringa oblata*, (2) *S. oblata* var. *affinis*, (3) *S. microphylla*, (4) *S. dilatata*, (5) *S. chinensis*, (6) *S. villosa*, and (7) *S. meyeri*.

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Methods

Test of the vitality of fresh pollens for different species

The index of the vitality of pollen was the dyeing rate or the sprouting rate. Dyeing reagent was 0.025% hypo methyl-blueness liquor (Liu 1985; Zhu 2000) and the sprouting culture medium was solid medium. Microscope was used to count the number of livelihood pollens. The repeated method of counting was adopted, and the three-visual microscopic field in each repeat was employed for every species. Finally, the mean and variance were calculated and tested.

Effect of temperature on the vitality and storage capability

At first, air-drying the pollens in shade, and then putting them into the different glass tubes. Secondly, plugging up the mouth of the tubes with the absorbent cotton, and then pasting the labels and putting them into three desiccators. Finally, keeping the desiccators separately in a shady area at a temperature of 15-25°C, in a refrigerator at 0-2°C, and in a refrigerator at –4-0°C, and then testing the vitality of pollens at regular intervals.

Results and discussion

Difference of vitality of fresh pollen of different species

By observing and testing, it was found that the vitality of fresh pollen of seven species had marked difference. The results of dyeing and sprouting were shown in Table 1.

Table 1. The difference of fresh pollen vitality in seven species

Species	Dyeing rate /%	Sprouting rate /%	Test F=0.05	F=0.01
Syringa oblata	49.82	41.56	c	BC
S. oblata var. *affinis*	33.80	27.83	d	C
S. microphylla	74.60	71.50	ab	AB
S. villosa	81.50	79.33	a	A
S. meyeri	70.24	58.96	b	B
S. dilatata	53.45	50.37	c	B
S. chinensis	51.62	50.86	c	B

Note: the same capital letter indicates that the difference of vitality of fresh pollen between species is not significant at the 0.01 levels and the different capital letter indicates that the difference between species is significant at the 0.01 levels. The small letter indicates the difference at the 0.05 levels.

From Table 1, the vitality of fresh pollen from high rate to low rate was listed as *S. villosa* (81.5%)> *S. microphylla* (74.6%)> *S. meyeri* (70.24%)> *S. dilatata* (53.45%)> *S. chinensis* (51.62%)> *S. oblata* (49.82%)>*S. oblata* var. *affinis* (33.8%). The results also showed that the differences in pollen vitality were not significant between the *S. microphylla* and *S. meyeri*, and the *S. oblata* and *S. chinensis* but very significant between *S. microphylla*, *S. villosa* and *S. chinensis*. These phenomena indicated that the species have far or close genetic relationships (Zang 1990). The *Syringa* was divided into four groups (subgenus) according to the genetic relationships. *S. chinensis* and *S. oblata* belong to *Vulgaris* group, and *S. microphylla* and *S. meyeri* belong to *Pubescens* group. Thus, their hereditary characters were very close and the difference in pollen vitality was very small. On the other hand, *S. microphylla*, *S. villosa* and *S. chinensis* belong to *Vulgaris* group, *Pubescens* group, and *Villosa* group, respectively. Therefore, the genetic relationships between them were more distant than that of the species belonging to the same group. The closer the genetic relationship of the species in *Syringa* was, the closer the pollen vitality of the species was.

Effect of temperature on the vitality of pollens

The pollen samples were stored separately at 15-25°C (room temperature), 0-2°C (refrigerator), and –4-0 °C (refrigerator) and the vitality of all the pollen samples was tested after different stored days

The pollens vitality of all species decreased rapidly at the temperature of 15-25°C (Table 2). In the fifth day, the pollen vitality was near to zero. The possible explanation is that the inner respiratory intensity was getting so strong that the pollens cannot survive at such temperature. This led to the nutrition of the pollen (the respiratory substrate) be dried up, meantime the temperature and humidity of the room made the pollen dehydrate and die.

Table 2. The change of pollen vitality of seven species under the room temperature in five days

Species	Pollen vitality (dyeing rate %)				
	1st day	2nd day	3rd day	4th day	5th day
Syringa oblata	49.82	38.48	10.87	3.94	0
S. oblata var. *affinis*	33.8	16.71	1.35	0	0
S. villosa	81.5	79.35	58.26	26.87	8.35
S. microphylla	74.6	66.39	38.51	8.66	2.34
S. chinensis	53.62	20.97	6.94	0	0
S. meyeri	70.24	59.23	21.36	5.76	1.04

At the temperature of 0-2 °C, the pollen vitality of all species was prolonged to half a month (Table 3) compared with samples under the temperature of 15-25 °C. In particular for the species of *S. microphylla* and *S. meyeri*, their pollen vitality could still reach 19.32% and 11.52% separately after being stored 60 days under this condition. Such pollen could still be used for pollination. The pollen of *S. oblata* var. *affiniis* was near to die after being stored 3 days at the temperature of 15-25 °C, but it could be preserved for 15 days under the temperature of 0-2 °C.

The pollens stored at the temperature of –4-0°C were defrosted before testing, two kinds of methods were adopted to defrost or melt the pollens. One was the quick defrosting method (the frosted pollens were putted into boiling water for melting), and the other was normal defrosting method (the pollens were kept at the normal temperature for slow melting). The change of pollen vitality

was shown as in Fig.1 and Fig.2.

Table 3. The change of vitality of pollens for seven species after stored different days under the temperature of 0-2℃

Species	Pollen vitality (dyeing rate %)					
	1day	5 days	10 days	15 days	30 days	60 days
Syringa. oblata	49.82	42	33.92	18 17	6.38	0
S. oblata var. affinis	33.8	26.39	23.65	4.88	3.19	0
S. villosa	81.5	75.42	69 96	37.65	16.47	9.16
S. microphylla	74.6	70.38	59.16	47.99	29.83	19.32
S. chinensis	53.62	39.93	18.85	12.14	5.46	2.02
S. meyeri	70.24	67	38.99	26.17	21.39	11.54

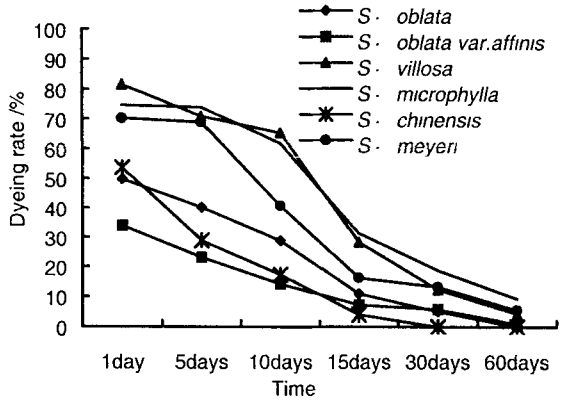

Fig.1 The change of pollen vitality of seven species treated by quick defrosting method after being frozen under –4-0℃

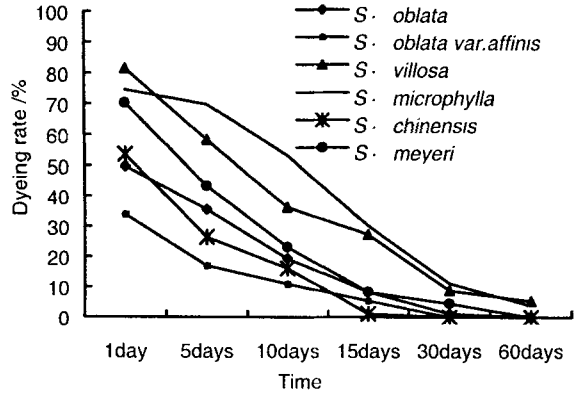

Fig.2 The change of pollen vitality of seven species treated by normal defrosting method after being frozen under –4-0℃

As shown in Fig.1, by using the quick defrosting to treat the pollens stored at the temperature of –4-0 °C for 10 days, the pollen vitality was still very high. The pollen vitality of *S. microphylla* and *S. meyeri* could reach 61.82% and 40.66%, respectively, which were higher than that of stored at the temperature of 0-2 °C for 10 days (59.16% and 38.99%). As shown in Fig.2, by using the normal defrosting to treat the pollens stored under the same condition, the pollen vitality was decreased rapidly.

When pollens were frozen over 10 days, the vitality of pollens of all seven species was very low no matter using which defrosting method for melting.

Storage capacity of fresh pollen of different species

By the above results, we found that t the storage capacity of different species from high to low was in order as: *S. microphylla* > *S. meyeri* > *S. villosa* > *S. oblata* > *S. chinensis* > *S. oblata* var. *affinis*. The pollen vitality of *S. microphylla* was the highest. It decreased very slowly, particularly for the pollen stored at the temperature of 0-2 °C, and it still kept a dyeing rate of 19.32% after stored 60 days (Table 3), which was still good for crossbreeding. After being frozen at the -4-0 °C for 30 days the pollen vitality of *S. microphylla* was 18.79% when the quick defrosting method was used. *S. meyeri* and *S. villosa* were in second order in respect to the storage capacity of pollen. The pollen vitalities of *S. meyeri* and *S. villosa* could reach 9.16% and 11.54% (Table 3), respectively, after being stored at the temperature of 0-2 °C for 60 days, and were 12.39% and 13.25% respectively after being frozen at the temperature of -4-0 °C for 30 days and defrosted by the quick defrosting method As for the other species, their pollens gradually lost their vitalities in 15 days and near to die after 30 days.

Conclusions and suggestions

Pollen vitality of the different species

The pollen vitality of different species from *Syringa* is different. The closer the genetic relationship between the species is, the closer the pollen vitality is. When we select the species for crossbreeding, we should select those who have higher pollen vitality as the male parents to insure a successful crossbreeding.

Effect of temperature on the life of pollen

The pollen vitality of all species decreased rapidly in the room temperature. The proper low temperature could prolong the life of pollen (Zhang 2000; Xue 2000). The vitality decreased with the prolonging of storage time, and the degressive speed was different with the difference of storage temperature. The most proper temperature for storing the pollen of *Syringa* is 0-2 °C, and the second is -4-0 °C. The pollen can also be preserved very well at the temperature of –4-0 °C, but it must be treated by the quick defrosting method.

Storage capacity of the pollen of different species

The storage capacity of pollen of different species has significant difference. The pollen of *S. microphylla* can be stored for 60 days at the temperature of 0-2°C, with a dyeing rate of 19.32. And the pollen of *S. meyeri* and *S. villosa* can also be stored about 60 days under such temperature condition, with a little lower dyeing rate, but the other species only can be stored in 15 days at most.

References

Gong Bangchu and Wang Jinfeng. 1997. Study on pollination biology in persimmon trees [J]. Forest Research, **10**(3): 237-243. (in Chinese)

Gu Shufen, Zhang Xudong, and Li Shuhua. 1998. The storage and vitality test of *Syringa* pollen [J]. Forestry Science & Technology, **23**(6): 18-19. (in Chinese)

Lai Huanlin, Chen Tianhua, Xu Jin, *et al.* 1994. Study on pollen vitality of clones from a Mason Pine seed orchard [J]. Forest Research, **7**(5): 555-560. (in Chinese)

Liang Jianping, Han Youzhi, Liang Shengfa, *et al.* 2000. Research on the vitality and storage characteristic of *Syringa* pollen [J]. Shanxi Forestry Science&Technology, **1**(1): 10-11. (in Chinese)

Li Dazhi, Xie Shenxi, Wei Yuerong, *et al.* 1999. Studies on pollen vitality of pears in Hunan [J]. Journal of Hunan Agricultural University, **25**(3): 184-187. (in Chinese)

Liu Wei, Gu Shufen, *et al.* 2000. Study on sexual hybridization of *Syringa* [J]. Bulletin Botanical Research, **20**(2): 207-211. (in Chinese)

Liu Wulin. 1985. The collection, storage and vitality test of pollen [J]. Chinese Bulletin of Botany, **3**(3): 8-12. (in Chinese)

Wang Qinli, Lu Longdou, Wu Xiaoqin, *et al.* 2002. Pollen preservation and vitality test [J]. Chinese Bulletin of Botany, **19**(3): 365-373. (in Chinese)

Wang Yan and Zhang Liming. 2002. Influences of storage temperatures on activated pollen ratio in *Ginkgo biloba* [J]. Journal of Hubei Agricultural College, **22**(3): 213-214, 218. (in Chinese)

Wang Yalin, Sheng Bingcheng, Yu Jing, *et al.* 1987. Study on pollen vitality and storage capacity in persimmon trees [J]. China Fruits, (2): 13-16. (in Chinese)

Wu Guoliang, Yang Zhihong, and Liu Qunlong. 1998. The biological characteristics of flowering and pollination in early lilac and its white flowered variety [J]. Journal of Beijing Forestry University, **20**(2): 118-120. (in Chinese)

Xue Miaonan, Liu Huaying, and Rao Furong. 2000. Study on low temperature preservation of Shatinyu's pollen [J]. GUIHAIA, **20**(4): 367-370. (in Chinese)

Zang Shuying and Fan Yinghan. 1993. The introduction of *Syringa* [J]. Plant Introduction and Acclimatization, (8): 41-54. (in Chinese)

Zang Shuying and Li Ronghui. 1992. The actuality and foreground of the introduction of *Syringa* [J]. Chinese Bulletin Botany, **9**(2): 30-33. (in Chinese)

Zang Shuying and Liu Gengxi. 1990. *Syringa* [M]. Beijing: China Forestry Publishing House. (in Chinese)

Zhang Yujin and Zhang Xingguo. 2000. Conservation of amorphophallus pollen at low temperature in liquid nitrogen [J]. Acta Horticulturae Sinica, **27**(2): 139-140. (in Chinese)

Zhao Wei. 1999. Newly strategies in develop the new variety of *Syringa* in Harbin [J]. Forest Science & Technology, **24**(2): 57-58. (in Chinese)

Zhu Liwu, Sun Wenping and Chen Jiayu. 2000. Study on pollen vitality and pollination of Plum Cultivar [J]. Journal of Anhui Agricultural University, **27**(3): 214-216. (in Chinese)